AMENDMENTS TO THE SPECIFICATION

The abstract is now presented on a separate sheet of paper.

Please replace the Title with the following Title:

Methods For Identification Of Identical Nucleic Acid Fragments From Different Nucleic Acid Populations

Please insert the following new paragraph on page 1, line 3:

CROSS REFERENCE TO RELATED APPLICATION

The present application is the national stage and claims, under 35 U.S.C. § 371, the benefit of PCT/EP00/02053, filed March 9, 2000, which claims priority to Federal Republic of Germany Application 199 11 130.8, filed March 12, 1999.

Please replace page 3, lines 18-26 with the following amended paragraph:

Disadvantages of familial linkage studies include the Expensive aspect (Many PCR's, allele scoring is labour intensive, fluorescent marker labelling); slow because although some multiplexing can be achieved high parallelization is not possible (no microsatellite DNA chips); statistical power limited to dissect small effects; results are dependent on allele frequencies and heterozygosity; extensive family collections with affected individuals are necessary (200-2000 individuals); IBD regions usually exten[[t]]d over large regions unsuitable for direct gene cloning, often 10-15 mega bases (low resolution).

Please replace page 9, lines 18-19 with the following amended paragraph:

A method [[if]] is also provided to identify genomic DNA regions that are relevant to pathological conditions or particular traits.

Please replace page 10, lines 22-28 with the following amended paragraph:

A method [[if]] is also provided to identify DNA regions that are relevant to pathological conditions or particular traits comprising hybridizing at least two nucleic acid populations from different sources having the particular trait or pathology, and separating the identical heterohybrids formed which contain DNA regions that are relevant to said pathological conditions or particular traits, wherein the nucleic acid populations comprise amplified and/or pre-selected nucleic acids.

Please replace page 13, lines 5-15 with the following amended paragraph:

In a particular embodiment, the restriction fragments can be selected prior to the subsequent ligation and/or amplification step. In particular, the restriction fragments can be size-selected to allow a uniform amplification of all fragments. Size selection may be performed on a gel or by any other technique. On an agarose gel, the restriction fragments are size separated in an electric field beside a size standard for orientation. Fragments in the preferred size range can be cut from the gel and be extracted from the agarose using standard methods (e.g. ~~gel extraction kit~~ "Quiaex II", a gel extraction kit from Quiagen AG, Germany). Size separation can also be achieved using column separation with a sieving material like polyacrylamide, sephadex etc.

Please replace page 22, line 9 – page 23, line 27 (Example 1) with the following paragraph:

Genomic DNA from at least two related individuals, with the same disease phenotype, is extracted by standard methods, e.g., phenol-chlorophorme extraction. The DNA[[']]s are separately cut with a restriction enzyme (e.g., *Bam* HI) to create restriction fragments with an average size around 4 kilobases. To these restriction fragments a solution containing short double stranded oligonucleotides (adaptors) is added. The adaptor molecules have sequence ends complementary to the restriction site sequences to allow ligation. The adaptors are then ligated to the restriction fragments from the genomic DNA[[']]s using a common ligase (e.g., T4 ligase). The sequence of the adaptors has been chosen in a way that: a) the sequence includes the recognition site for *mut* HL, b) adapter dimers formed through autoligation of

two adaptor molecules are self-complementary and don't compete for primers with the genomic ligation products during PCR. The adaptor carrying fragments are then, separately for each individual, amplified by PCR using primers that are complementary to a part of the adaptor sequence and that carry unique 5' ends. After several rounds of amplification the PCR products of different individuals differ by their ends in respect to each other. The amplification products are then mixed, heat denatured and allowed to re-anneal using stringent hybridisation conditions (Casna et al. (1986) genomic analysis II, isolation of high molecular weight heteroduplex DNA following methylase protection and formamide PERT [[*h*]]*Hybridization Nucleic Acids Res.* 14: 7285-7303). This results in the formation of heteroduplexes from the DNA[[']]s from different sources (individuals) with forked (single stranded) ends because of the non-complementarity of the primer sequences. In addition homoduplexes are formed by renaturation between the strands of one individu[[l]]al with itself. These homoduplexes are blunt-ended. To this mixture a solution containing *exo* III (or an equivalent 3' recessed or blunt-end specific exonuclease) exonuclease is added. The exonuclease digests the blunt ended homoduplexes but not the heteroduplexes with their 3' overhang, creating big single stranded gaps in the homoduplex fragments. These can be eliminated from the reaction mix through binding to a single strand specific matrix (e.g., BND cellulose beads). The remaining heteroduplexes comprise a pool of 100% identical fragments and fragments with base pair mismatches (non-IBD fragments). A solution containing the mismatch repair enzymes *mut* SHL is added to the mix resulting in the nicking of mismatched heteroduplexes at a specific recognition site (GATC). These nicks are further digested by adding *exo* III (or an equivalent 3' recessed or blunt-end specific exonuclease) exonuclease to the reaction mix, creating big single stranded gaps in the homoduplex fragments. These can be eliminated from the reaction mix through binding to a single strand specific matrix (e.g., BND cellulose beads). The remaining fragments in the reaction mix constitute a pool of 100% identical DNA hybrids formed between the DNA[[']]s of different individuals comprising the loci responsible for the disease phenotype. These fragments can be detected and identified (e.g., by hybridisation to a DNA array representing the whole human genome). Comparison of the signals from a number of experiments in different families with the same disease phenotype allows the identification of the regions linked to disease (disease specific genome haplotype).

Please replace page 24, line 1 – page 25, line 26 (Example 2) with the following paragraph:

One aim in modern agricultural animal breeding is the selection for or against certain quantitative trait phenotypes (e.g., muscle mass, milk quantity, concentration of caseine in milk for cheese production etc.). The genetic mechanisms leading to a trait are often complex with several loci implicated. These loci can be identified using our procedure. In this example genomic DNA from different animals concordant for a trait of interest (e.g., higher than average caseine concentration in milk) is restricted using a restriction endonuclease that produces on average fragments around 4 kilobases (e.g., *Bam* HI). To these restriction fragments a solution containing short double stranded oligonucleotides (adaptors) is added. The adaptor molecules have sequence ends complementary to the restriction site sequences to allow ligation. The adaptors are then ligated to the restriction fragments from the genomic DNA[[']]s using a common ligase (e.g., T4 ligase). The sequence of the adaptors has been chosen in a way that: a) the sequence includes the recognition site for *mut* HL, b) adapter dimers formed through autoligation of two adaptor molecules are self-complementary and don't compete for primers with the genomic ligation products during PCR. The adaptor carrying fragments are then separately amplified by PCR using primers that are complementary to a part of the adaptor sequence but that carry unique 5' ends. After several rounds of amplification the PCR products from the DNA[[']]s of different animals differ by their ends in respect to each other. The amplification products are then mixed, heat denatured and allowed to re-anneal using stringent hybridisation conditions (Casna et al. (1986) genomic analysis II, isolation of high molecular weight heteroduplex DNA following methylase protection and formamide PERT [[*h]]Hybridization Nucleic Acids Res.* 14: 7285-7303). This results in the formation of heteroduplexes between the DNA[[']]s from different animals, with forked (single stranded) ends because of the non-complementarity of the primer sequences. In addition homoduplexes are formed by renaturation between the strands of a given animal with itself. These homoduplexes are blunt-ended. To this mixture a solution containing *exo* III (or an equivalent 3' recessed or blunt-end specific exonuclease) exonuclease is added. The exonuclease digests the blunt ended homoduplexes but not the heteroduplexes with their 3' overhang, creating big single stranded gaps in the homoduplex fragments. These can be eliminated from the reaction mix through binding to a single strand specific matrix (e.g., BND cellulose beads). The remaining heteroduplexes comprise a pool of 100% identical fragments and fragments with base pair mismatches (non-IBD fragments).

A solution containing the mismatch repair enzymes *mut* SHL is added to the mix resulting in the nicking of mismatched heteroduplexes at a specific recognition site (GATC). These nicks are further digested by adding *exo* III (or an equivalent 3' recessed or blunt-end specific exonuclease) exonuclease to the reaction mix, creating big single stranded gaps in the homoduplex fragments. These can be eliminated from the reaction mix through binding to a single strand specific matrix (e.g., BND cellulose beads). The remaining fragments in the reaction mix constitute a pool of 100% identical DNA hybrids formed between the DNA[[']]s from different animals comprising the loci responsible for the quantitative trait of interest. These can be hybridised against an array containing a representative selection of sequences covering the whole genome of the animal. As in this case non-related animals can be used to identify the QTL's because the IBD regions should be small, i.e., a very limited number of experiments should be necessary (only one in the best case) to identify the genes responsible for the trait. The introduction of a control animal discordant for the trait of interest can further enhance the resolution of the system.

Please replace page 25, line 30 – page 27, line 27 (Example 3) with the following paragraph:

Depending on the complexity and heterogeneity of a disease phenotype, the locus definition after a GMS experiment as described in example 1 may vary between several kilobases and some megabases. In the latter case further experiments must be carried out to decrease the genetic interval in which the disease gene is located. The inventive procedure can also be used to fine map the gene(s) of interest. DNA from different non-related individuals that have been shown to be linked to the same disease loci is extracted and digested by a suitable restriction endonuclease (e.g., 4 base recognition site cutter) to produce well length defined fragments. To these restriction fragments a solution containing short double stranded oligonucleotides (adaptors) is added. The adaptor molecules have sequence ends complementary to the restriction site sequences to allow ligation. The adaptors are then ligated to the restriction fragments from the genomic DNA[[']]s using a common ligase (e.g., T4 ligase). The sequence of the adaptors has been chosen in a way that: a) the sequence includes the recognition site for *mut* HL, b) adapter dimers formed through autoligation of two adaptor molecules are self-complementary and don't compete for primers with the genomic ligation products during PCR. The adaptor carrying fragments are then, separately for each individual, amplified by PCR using primers that are complementary to a part of the

adaptor sequence and that carry unique 5' ends. After several rounds of amplification the PCR products of different individuals differ by their ends in respect to each other. The amplification products are then mixed, heat denatured and allowed to re-anneal using stringent hybridisation conditions (Casna et al. (1986) genomic analysis II, isolation of high molecular weight heteroduplex DNA following methylase protection and formamide PERT [[*h*]]*Hybridization Nucleic Acids Res.* 14: 7285-7303). Depending on restrictions for the choice of the unique 5' ends for the primers, the amplification products of several individuals can be mixed, enhancing the resolution. The mixing of the PCR fragments results in the formation of heteroduplexes from the DNA[[']]s from different sources (individuals) with forked (single stranded) ends because of the non-complementarity of the primer sequences. In addition homoduplexes are formed by renaturation between the strands of one individu[[l]]al with itself. These homoduplexes are blunt-ended. To this mixture a solution containing *exo* III (or an equivalent 3' recessed or blunt-end specific exonuclease) exonuclease is added. The exonuclease digests the blunt ended homoduplexes but not the heteroduplexes with their 3' overhang, creating big single stranded gaps in the homoduplex fragments. These can be eliminated from the reaction mix through binding to a single strand specific matrix (e.g., BND cellulose beads). The remaining heteroduplexes comprise a pool of 100% identical fragments and fragments with base pair mismatches. A solution containing the mismatch repair enzymes *mut* SHL is added to the mix resulting in the nicking of mismatched heteroduplexes at a specific recognition site (GATC). These nicks are further digested by adding *exo* III (or an equivalent 3' recessed or blunt-end specific exonuclease) exonuclease to the reaction mix, creating big single stranded gaps in the homoduplex fragments. These can be eliminated from the reaction mix through binding to a single strand specific matrix (e.g., BND cellulose beads). The remaining fragments in the reaction mix constitute a pool of small 100% identical DNA hybrids formed between the DNA[[']]s of different individuals comprising the loci responsible for the disease phenotype. As there is virtually no IBD between these individuals only a very small number of relatively short fragments should be identical (this is basically a very efficient way to search for allelic association). A dense locus specific array of DNA sequences can be used to detect and identify sequences within the pool of identical DNA[[']]s. As the sequences of the array are known they can be used to directly sequence the fragments from the GMS procedure to identify open reading frames (ORF's) and the genes of interest.

Please replace page 27, line 31 – page 29, line 12 (Example 4) with the following paragraphs:

Genomic DNA from at least two related individuals, with the same disease phenotype, is extracted by standard methods, e.g., phenol-chlorophorme extraction. The DNA[[']]s are separately cut with a restriction enzyme (e.g., *Bam* HI) to create restriction fragments with an average size around 4 kilobases. To these restriction fragments a solution containing short double stranded oligonucleotides (adaptors) is added. The adaptor molecules have sequence ends complementary to the restriction site sequences to allow ligation. The adaptors are then ligated to the restriction fragments from the genomic DNA[[']]s using a common ligase (e.g., T4 ligase). The sequence of the adaptors has been chosen in a way that: a) the sequence includes the recognition site for *mut* HL, b) adapter dimers formed through autoligation of two adaptor molecules are self-complementary and don't compete for primers with the genomic ligation products during PCR. The adaptor carrying fragments are then, separately for each individual, amplified by PCR using primers that are complementary to a part of the adaptor sequence and that carry unique 5' ends. After several rounds of amplification the PCR products of different individuals differ by their ends in respect to each other. The amplification products are then mixed, heat denatured and allowed to re-anneal using stringent hybridisation conditions (Casna et al. (1986) genomic analysis II, isolation of high molecular weight heteroduplex DNA following methylase protection and formamide PERT [[*h*]]*Hybridization Nucleic Acids Res.* 14: 7285-7303). This results in the formation of heteroduplexes from the DNA[[']]s from different sources (individuals) with forked (single stranded) ends because of the non-complementarity of the primer sequences. In addition homoduplexes are formed by renaturation between the strands of one individu[[l]]al with itself. These homoduplexes are blunt-ended. To this mixture a solution containing *exo* III (or an equivalent 3' recessed or blunt-end specific exonuclease) exonuclease is added. The exonuclease digests the blunt ended homoduplexes but not the heteroduplexes with their 3' overhang, creating big single stranded gaps in the homoduplex fragments. These can be eliminated from the reaction mix through binding to a single strand specific matrix (e.g., BND cellulose beads). The remaining heteroduplexes comprise a pool of 100% identical fragments and fragments with base pair mismatches (non-IBD fragments).

A solution containing the mismatch recognizing protein *mut* S is added to the reaction mix. *Mut* S binds to the mismatched DNA at the site of the mismatch. The protein/DNA complex is then eliminated from the reaction mix by specific binding of *mut* S to a matrix (e.g., antibody carrying column, protein binding membrane). This procedure om[[m]]its the *mut* LH nicking steps and the second *exo* III digestion as well as the need for a single strand binding matrix to eliminate the products resulting from the exonuclease digestion. The remaining identical DNA heteroduplex fragments can be detected and identified as pointed out in example 1.